

User Bulletin

GeneMapper® ID Software

November 30, 2004

SUBJECT: Installation Procedures and New Features for
GeneMapper® ID Software v3.2

In This User Bulletin

This user bulletin includes the following topics:

Section 1 Installation Procedures	3
Section 2 New Features: All AmpF_{STR} Kits	17
Section 3 New Features and Procedures: Yfiler Kit	25
Appendix A Verification Testing	41
Appendix B Troubleshooting the Installation	57

Overview

GeneMapper® ID Software v3.1 has been upgraded to v3.2. This user bulletin:

- Provides GeneMapper ID Software v3.2 installation requirements and procedures
- Describes the new features in GeneMapper ID Software v3.2 and, where applicable, provides procedures for using them
- Describes verification testing of GeneMapper ID Software v3.2

Section 1 Installation Procedures

This section covers:

Supported Instruments and AmpFSTR Kits 4

Installation Requirements 6

Installation Procedures 10

Supported Instruments and AmpF/STR Kits

Instruments GeneMapper ID Software v3.2 is compatible with the instruments, operating systems, and Data Collection software listed in the table below. Compatibility is defined as the ability of the GeneMapper ID software to perform at least one of the following functions:

- A. Analyze sample files generated from the instrument
- B. Coexist with the instrument's Data Collection software
- C. Run concurrently with the instrument's Data Collection software
- D. Work with the automation feature (that is, autoanalysis) in the instrument's Data Collection software

Instrument	Computer Operating System	Data Collection Software	Functions (as listed above)			
			A	B	C	D
ABI PRISM® 377 DNA Sequencer	Macintosh® a	2.6	X	—	—	—
	Microsoft® Windows NT®	3.0	X	X	X	—
ABI PRISM® 310 Genetic Analyzer	Macintosh® a	2.1	X	—	—	—
	Microsoft® Windows NT®	v3.0	X	X	X	—
	Microsoft® Windows® 2000	v3.0	X	X	X	—
ABI PRISM® 3100 Genetic Analyzer	Microsoft® Windows NT®	v1.1	X	—	—	—
	Microsoft® Windows® 2000	v2.0	X	X	X	X
ABI PRISM® 3100-Avant Genetic Analyzer	Microsoft® Windows NT®	v1.0	X	—	—	—
	Microsoft® Windows® 2000	v2.0	X	X	X	X

a. Data generated from a 310 Genetic Analyzer or 377 DNA Sequencer using a Macintosh® platform must be converted to a Microsoft® Windows®-based format before they can be used with the GeneMapper ID software. For information on converting Macintosh® sample files, refer to the GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide (PN 4338775).

Chemistry Kits GeneMapper *ID* Software v3.2 is specifically designed to work with the following AmpFℓSTR® kits:

- AmpFℓSTR® Yfiler™ PCR Amplification Kit
- AmpFℓSTR® Identifiler® PCR Amplification Kit
- AmpFℓSTR® SGM Plus® PCR Amplification Kit
- AmpFℓSTR® Profiler Plus® ID PCR Amplification Kit
- AmpFℓSTR® Profiler Plus® PCR Amplification Kit
- AmpFℓSTR® COfiler® PCR Amplification Kit
- AmpFℓSTR® SEfiler™ PCR Amplification Kit
- AmpFℓSTR® Profiler® PCR Amplification Kit
- AmpFℓSTR Blue™ PCR Amplification Kit
- AmpFℓSTR Green™ I PCR Amplification Kit

Installation Requirements

Computer Specifications

The computer specifications for GeneMapper *ID* Software v3.2 are listed in the table below. Please note the following:

- The *Minimum Requirements* column lists the lowest specifications that permit the installer to install the GeneMapper *ID* software. The minimum requirements may not provide optimal performance.
- The *Recommended Requirements* column lists the requirements that are recommended by Applied Biosystems.

System Component	Minimum Requirements	Recommended Requirements
Computer	• Intel Pentium® III processor, 540 MHz • 256 MB of RAM • 2-GB hard drive (free space)	• Intel Pentium® IV processor, ≥2.8 GHz • 512 MB of RAM • 40-GB EIDE hard drive • 20/48X IDE CD-ROM • 10/100 NIC with RWV (internal)
Monitor	800 × 600 pixels size 17-inch monitor	1024 × 768 or higher pixels size 19-inch or larger monitor
Operating System	Microsoft Windows NT® version 4.0 (Service Pack 5 or higher)	• Microsoft Windows® 2000 Professional (Service Pack 4) • Microsoft Windows® XP Professional (Service Pack 1)
Ethernet Capability	• Network card for Oracle® installation • TCP/IP must be installed prior to Oracle installation	• Network card for Oracle® installation • TCP/IP must be installed prior to Oracle installation

Special Considerations

- The GeneMapper *ID* software does not run correctly on dual-processor Pentium® computers. A single-processor computer is required.

- GeneMapper *ID* Software v3.2 runs on Windows NT[®], Windows[®] 2000, and Windows[®] XP platforms only. Conversion utilities are included for analyzing data from Macintosh[®]-based sample files. For conversion procedures, refer to the *GeneMapper[™] ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775).
- The GeneMapper *ID* Software v3.2 automated installer operates only on computers running Microsoft Internet Explorer. If you are using another browser, you will need to manually launch the installer; instructions are provided in [step 2 on page 12](#).

Compatibility with Oracle Databases

The GeneMapper *ID* software uses an Oracle[®] database. GeneMapper *ID* Software v3.2 is compatible only with the Oracle database installed with ABI PRISM[®] 3100/3100-*Avant* Genetic Analyzers Data Collection Software v2.0.

Be sure there is no other Oracle client or Oracle server on the computer. GeneMapper *ID* Software v3.2 is not supported on any other Oracle client or server configurations.

Special Considerations

- The version of the Oracle database in GeneMapper *ID* Software v3.2 is an embedded license for use by five named users.

IMPORTANT! To accommodate more than five users, additional GeneMapper *ID* software or Oracle database licenses must be purchased.

- TCP/IP has to be installed before installing GeneMapper *ID* Software v3.2.

**Compatibility
with 3100/3100-
Avant Genetic
Analyzers**

The table below describes compatibility of GeneMapper *ID* Software v3.2 with the Data Collection software for the ABI PRISM® 3100/3100-*Avant* Genetic Analyzers.

3100 Genetic Analyzer Data Collection Version	3100-Avant Genetic Analyzer Data Collection Version	Compatible with Instrument Computer?
1.1	—	No. ^a
—	1.0	
2.0	2.0	Yes

a. GeneMapper *ID* Software v3.2 can only exist with the Oracle database installed by 3100/3100-Avant Genetic Analyzer Data Collection Software v2.0.

IMPORTANT! To install GeneMapper *ID* Software v3.2 on a computer connected to a 3100/3100-Avant Genetic Analyzer that uses Data Collection Software v2.0, the Data Collection software must be running. If the Data Collection software is not running, the GeneMapper *ID* software does not register with the Data Service software.

**Login
Requirements**

When installing GeneMapper *ID* Software v3.2, you must:

- Have Administrator privileges on the local computer (that is, have complete and unrestricted access to the local computer)
- Log in to the local computer (not a network domain)
- Be sure there is no other Oracle client or Oracle server on the computer. GeneMapper *ID* Software v3.2 is not supported on these configurations.

PDF File Reader To open the user documentation included on the *GeneMapper® ID Software v3.2* installation CD, use a program that reads PDF files, such as the Adobe Acrobat® Reader software.

The GeneMapper *ID Software v3.2* installer does not install Acrobat Reader. If you do not have a program that reads PDF files, you can download Acrobat Reader from either the Adobe or Applied Biosystems websites:

- www.adobe.com
- www.appliedbiosystems.com/support/software.

Installation Procedures

What Gets Installed

The installer on the *GeneMapper® ID Software v3.2* CD installs:

- GeneMapper® ID Software v3.2
- Oracle® database
- Panel folder that contains AmpFSTR® kit panels and bins
- Microsatellite and SNP genotyping example data
- Electronic (PDF) versions of the user guide and tutorial
- Size standard folder that contains various size standards
- Default plot and table settings

Before You Begin the Installation

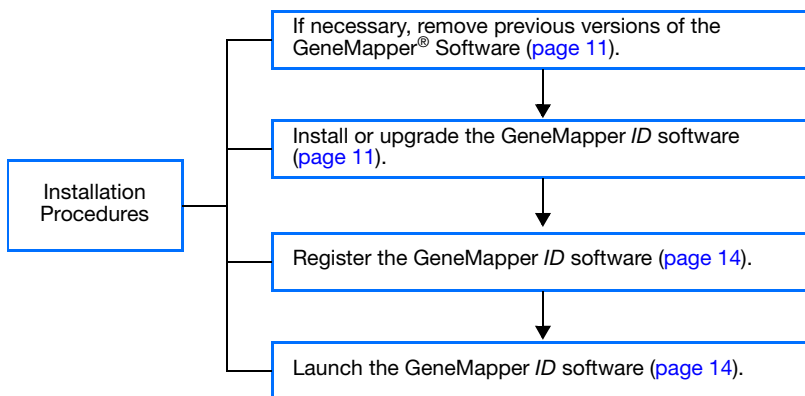
Before you begin the installation, be sure all requirements are met. See the references listed below.

- [“Computer Specifications” on page 6](#)
- [“Compatibility with Oracle Databases” on page 7](#)
- [“Compatibility with 3100/3100-Avant Genetic Analyzers” on page 8](#)
- [“Login Requirements” on page 8](#)
- [“PDF File Reader” on page 9](#)

Installation Time

The total time required to install the GeneMapper ID software is approximately 60 minutes. This time may vary, depending on your system configuration.

Procedure Flowchart



Remove Non-Validated GeneMapper Software (If Necessary)

If you have either GeneMapper® Software v3.0 or v3.5 on your computer, you must remove it before installing the GeneMapper *ID* software.

To remove GeneMapper software v3.0 or v3.5:

1.	Log in to the local computer as an Administrator.
2.	Select Start > Control Panel to open the Control Panel window.
3.	Double-click Add or Remove Programs to open the Add or Remove Programs dialog box.
4.	Select the GeneMapper software program, then follow the prompts to uninstall/remove the GeneMapper software.
5.	If prompted to do so, restart the computer.
6.	Continue with “Install the Software” below.

Install the Software

To install GeneMapper *ID* Software v3.2:

1.	Log in to the local computer as an Administrator. IMPORTANT! If you do not have Administrator privileges, log off of the computer and log back in as a user with Administrator privileges.
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To install GeneMapper ID Software v3.2: (continued)

2. Insert the *GeneMapper® ID Software v3.2* CD into the CD-ROM drive.

The installer launches automatically, displaying the Main Menu.

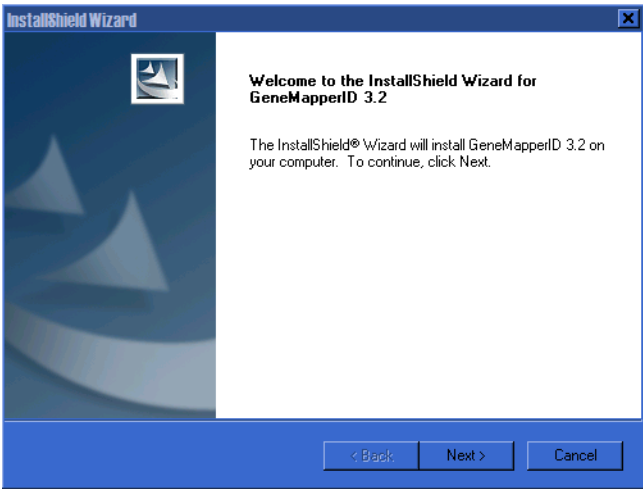


Note: If the installer does not launch automatically:

- a. On the desktop, right-click the **My Computer** icon and select **Explore** to open the My Computer window.
 - b. Double-click the CD drive to open the **GeneMapper ID v3.2** folder.
 - c. Double-click the **Setup.exe** file to launch the installer.
3. Click **Installation Readme** to view the InstallationReadme.txt file on the *GeneMapper® ID Software v3.2* CD.

Note: The InstallationReadme.txt file may contain installation information that is more current than the information in this user bulletin.

To install GeneMapper ID Software v3.2: (continued)

4.	Click Install GeneMapper® ID Software v3.2. to start the InstallShield® Wizard. 
5.	Follow the onscreen instructions to install the software. IMPORTANT! During installation, DOS commands are executed and the DOS window is opened. Do not delete, close, or click in the DOS window. If you click in it, press the Esc key.
6.	When the installation is complete, click Finish.
7.	If prompted to do so, restart the computer.
8.	Continue with “Register the Software” on page 14.

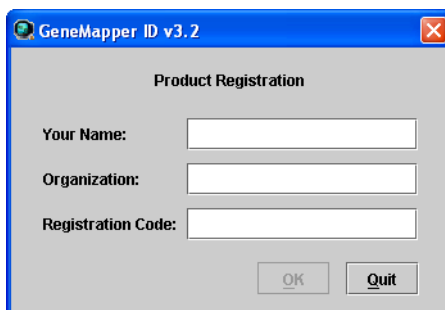
Register the Software

To register GeneMapper ID Software v3.2:

1.	Locate the registration code on the <i>Getting Started Card</i> in the software package.
2.	IMPORTANT! Keep the registration code in a place where you can easily retrieve it. You will need the registration code the first time you start the GeneMapper ID software (below). In addition, if you need to reinstall the software at any time, you are prompted for the registration code again.
3.	Complete the registration card and return it to Applied Biosystems.
4.	Continue with “Start the Software” below.

Start the Software

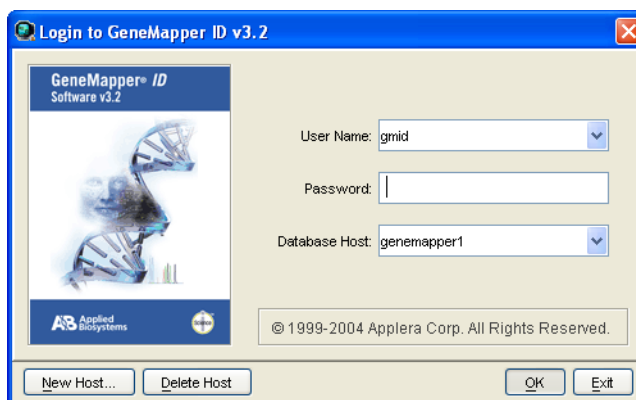
To start GeneMapper ID Software v3.2 for the first time:

1.	Log in to the local computer.
2.	On the desktop, click Start > Programs > Applied Biosystems > GeneMapper > GeneMapper ID v3.2. The first time you start the GeneMapper ID software, the Product Registration dialog box opens.  Note: If the GeneMapper ID software fails to start, see “Troubleshooting the Installation” on page 57.

To start GeneMapper ID Software v3.2 for the first time:

3. Register the software:
 - a. In the Product Registration dialog box, enter your name, organization, and registration code.
 - b. Click **OK**.

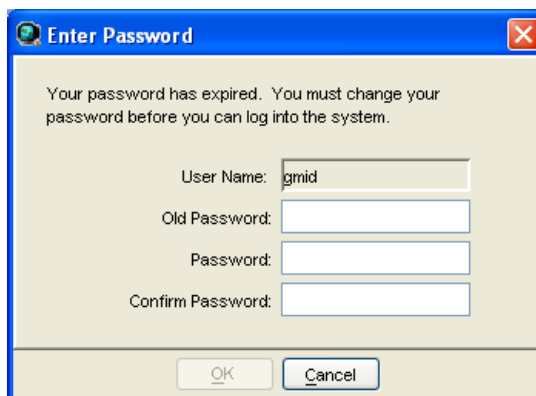
The software verifies the registration code, then displays the Login dialog box.



To start GeneMapper ID Software v3.2 for the first time:

4. The first time you launch the GeneMapper ID software, complete the Login dialog box as follows:
 - a. Leave the User Name as **gmid** and leave the Password field blank.
 - b. From the Database Host drop-down list, select the local computer (that is, select the name of the computer you are currently working on).
 - c. Click **OK**.

The Enter Password dialog box opens.



5. Create your password:
 - a. Leave the Old Password field blank.
 - b. In the Password field, enter a new, user-defined password.
 - c. Confirm the password.
 - d. Click **OK**.

The GeneMapper ID software starts.

Section 2 New Features: All AmpF ℓ STR Kits

This section covers:

New Features Summary	17
Export Combined Table Format	18
Changes to the Electropherogram Displays	22

New Features Summary

[Table 1](#) below provides a brief summary of the new features included in GeneMapper *ID* Software v3.2. For more detailed information, see the pages referenced in the table.

Table 1 New features summary

Feature	Description	See Page
Export Combined Table format	When exporting from the Samples view, you can now export samples that do not pass sizing along with samples that pass sizing. This feature combines columns from the sample table and the genotype table and exports them as a single table.	18
	- You now have two display options when exporting samples: - One line per marker - One line per sample (This is similar to the Make Allele table in the Genotyper[®] Software.)	
Changes to the electropherogram displays	The software now provides the option to display labeled peak assignments for all size standards. This allows you to quickly identify peaks visually and perform a size precision test. The labeled peak assignments are printable.	37
	When switching from the “align by base pair” to the “align by data point” views for the X-axis, the labels associated with the peaks are now retained in both views.	

Export Combined Table Format

GeneMapper ID Software v3.2 can export data in the *Export Combined Table* format. This new format:

- Allows you to track *all* samples in an electrophoresis run. That is, when exported from the Samples view, samples that do not pass sizing are exported along with samples that pass sizing.
- Provides two display options: one line per marker and one line per sample

Size Quality Status in the Samples View

As shown in [Figure 1](#) below, the size quality status is displayed in the SQ column of the Samples view:

- Samples that do not pass sizing are flagged with a red circle.
- Samples that pass sizing are flagged with a green square.

When you create an Export Combined Table, both the passing and low-quality samples are exported together, as shown in [Figure 2](#) on [page 19](#) and [Figure 3](#) on [page 20](#). The procedure for creating an Export Combined Table is on [page 20](#).

Status	Sample File	Sample Name	Sample Type	Specimen Cate	Analysis Method	Panel	Size Standard	SQ
1	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	None	●
2	A111250.fsa	1250	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	None	●
3	A12ntc.fsa	ntc	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	None	●
4	A1500500.fsa	500500	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	None	●
5	A2500250.fsa	500250	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	None	●
6	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
7	A450002.fsa	50002	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
8	A550031.fsa	50031	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
9	A65000.fsa	5000	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
10	A7ladder.fsa	ladder	Allelic Ladder	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
11	A8125125.fsa	125125	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
12	A912502.fsa	12502	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
13	B1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
14	B111250.fsa	1250	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
15	B1500500.fsa	500500	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
16	B2500250.fsa	500250	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
17	B3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
18	B450002.fsa	50002	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
19	B550031.fsa	50031	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
20	B65000.fsa	5000	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
21	B7ladder.fsa	ladder	Allelic Ladder	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
22	B8125125.fsa	125125	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■
23	B912502.fsa	12502	Sample	no export	AmpFISTR_Yfiler_Adva	Yfiler_v1	CE_05_HID_05500	■

These samples do not pass sizing.

These samples pass sizing.

Figure 1 Size quality status

Two Export Display Options

Two display options are available to export samples that do not pass sizing with samples that pass sizing:

- One line per marker

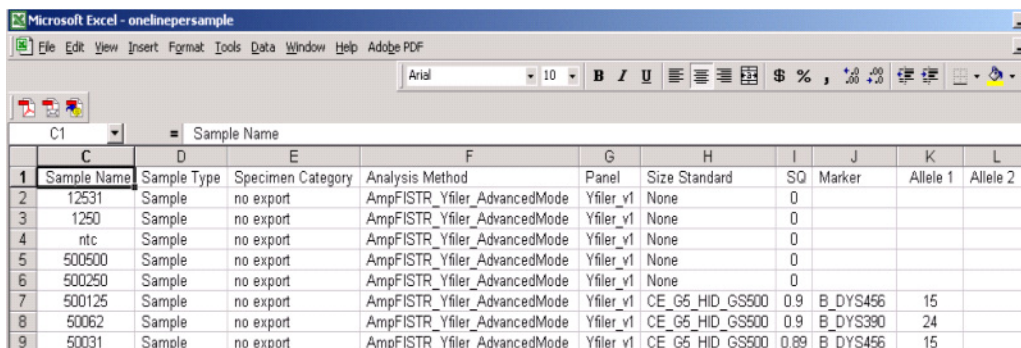
This option displays information for each marker on a separate line for a given sample. In [Figure 2](#) below for example, lines 2 through 15 display the information for each marker in sample file A1012531.fsa.

	B	C	D	E	F	G	H	I	J
	Sample File	Sample Name	Sample Type	Specimen Category	Analysis Method	Panel	Size Standard	SQ	Marker
2	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	B_DYS456
3	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	B_DYS3891
4	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	B_DYS390
5	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	B_DYS3891
6	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	G_DYS456
7	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	G_DYS190
8	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	G_DYS385
9	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	Y_DYS393
10	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	Y_DYS391
11	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	Y_DYS439
12	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	Y_DYS635
13	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	Y_DYS392
14	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	R_Y_GATA
15	A1012531.fsa	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0	R_DYS437
82	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS456
83	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS3891
84	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS390
85	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS3891
86	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	G_DYS456
87	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	G_DYS190
88	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	G_DYS385
89	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	Y_DYS393
90	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	Y_DYS391
91	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	Y_DYS439
92	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	Y_DYS635
93	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	Y_DYS392
94	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	R_Y_GATA
95	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	R_DYS437
96	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	R_DYS456
97	A3500125.fsa	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	R_DYS448
98	A450062.fsa	50062	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS390
99	A450062.fsa	50062	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS3891

Figure 2 One line per marker

- One line per sample

This option displays the information for each sample on just one line. In [Figure 3](#) below for example, line 2 displays information for Sample Name 12531. The subsequent lines display information for different samples.



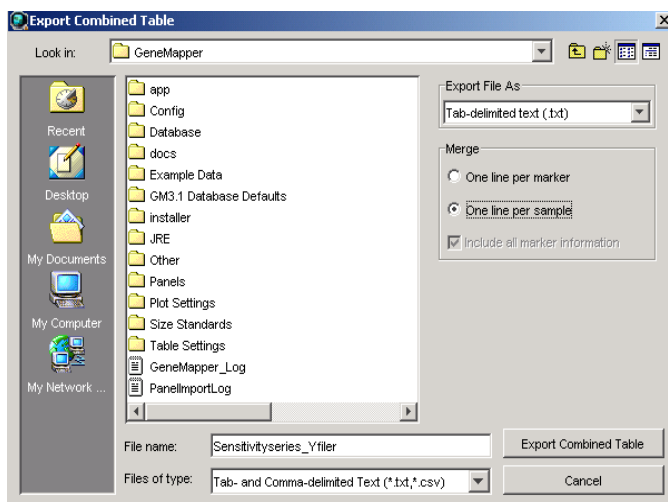
	C	D	E	F	G	H	I	J	K	L
	Sample Name	Sample Type	Specimen Category	Analysis Method	Panel	Size Standard	SQ	Marker	Allele 1	Allele 2
1	12531	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0			
2	1250	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0			
3	ntc	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0			
4	500500	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0			
5	500250	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	None	0			
6	500125	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS456	15	
7	50062	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.9	B_DYS390	24	
8	50031	Sample	no export	AmpFISTR_Yfiler_AdvancedMode	Yfiler_v1	CE_G5_HID_GS500	0.89	B_DYS456	15	

Figure 3 One line per sample

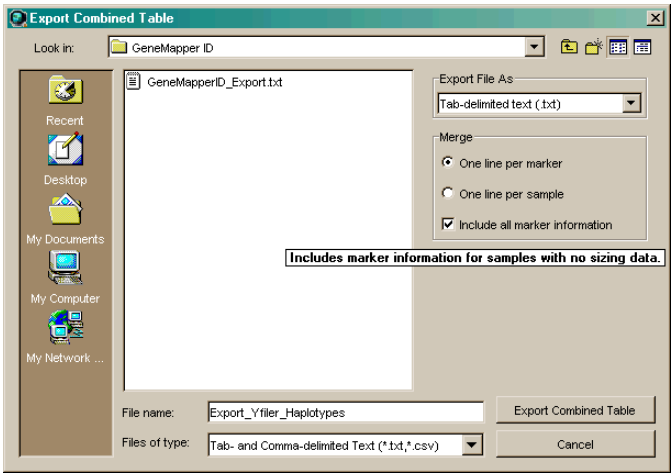
Creating an Export Combined Table

To create an Export Combined Table:

1. After analyzing your samples, go to the toolbar and select **File > Export Combined Table** to open the Export Combined Table window.



To create an Export Combined Table:

2.	From the Look in drop-down list, select an appropriate destination folder for the Export Combined Table.
3.	From the Export File As drop-down list, select a file type.
4.	From the Merge panel, select the format style: • One line per marker, <i>or</i> • One line per sample Note: See page 19 and page 20 for an example of each format.
5.	If you selected One line per marker in step 4, select/deselect the Include all marker information checkbox, as necessary. As shown in the pop-up text below, if you select this option, marker information for samples with no sizing data will be included in the Export Combined Table. 
6.	Type in a File name .
7.	From the Files of type drop-down list, select the appropriate file type/extension.
8.	Click Export Combined Table .

Changes to the Electropherogram Displays

In GeneMapper ID Software v3.2, two changes have been made to the electropherogram displays:

- Labeled peak assignments can be displayed for all size standards
- Labels are retained in both the “align by base pair” and “align by data point” views for the X-axis

Labeled Peak Assignments for All Size Standards

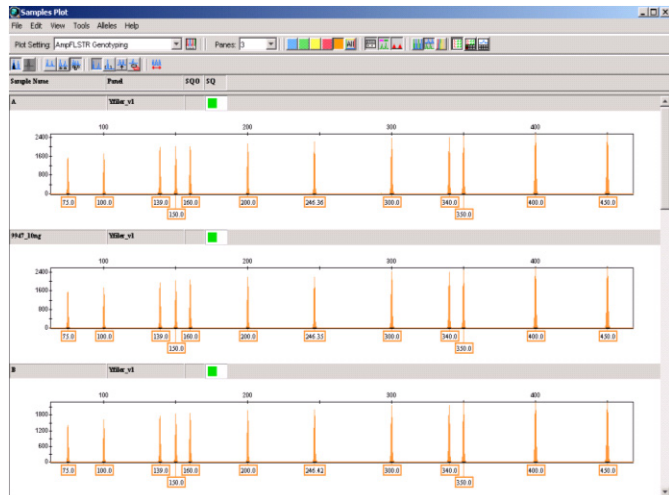
GeneMapper ID Software v3.2 now provides the option to display labeled peak assignments for all size standards in the electropherograms. (That is, all defined size standard peaks are labeled in the electropherogram per the size standard definitions). This allows you to quickly identify peaks visually and perform a size precision test.

To display peak assignments for all size standards:

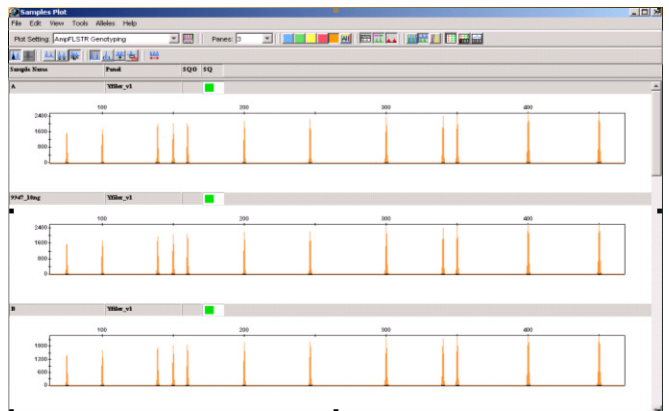
1.	In the Samples view, highlight all sample files in the project.
2.	Click the Display Plots icon.
3.	From the Plot Setting drop-down list, select AmpFLSTR Genotyping .
4.	Hide all dyes except the size standard dye (that is, red for ROX™ dye or orange for LIZ® dye).
5.	Select View > Show Size Standard Label .
6.	Verify that all values for the 250-bp fragment peaks are within ± 0.5 bp.

To display peak assignments for all size standards: *(continued)*

7. If desired, select **File > Print** to print the electropherogram plots. The examples below show both labeled and unlabeled peak assignments.



Labeled peak assignments



Unlabeled peak assignments

Labels Retained in Base Pair and Data Point Views

The X-axis can be displayed in either base pairs or data points. In GeneMapper ID Software v3.1, when switching from the “align by base pair” to the “align by data point” views for the X-axis, the labels associated with the peaks were not retained in both views. In GeneMapper ID Software v3.2, the labels are automatically retained in both views, as shown below.

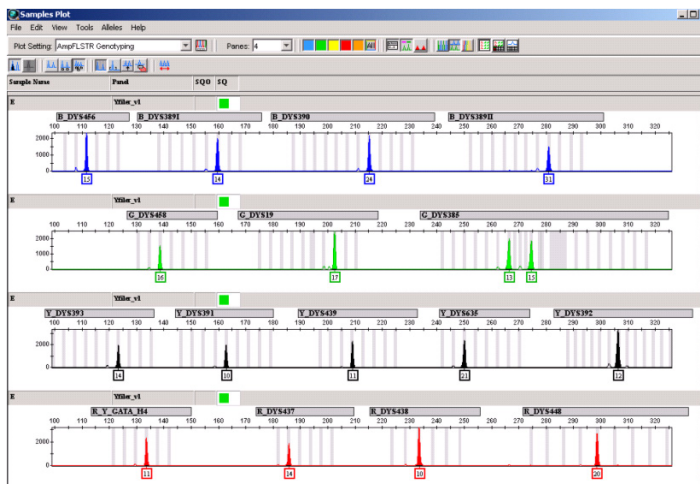


Figure 4 Align by base pair

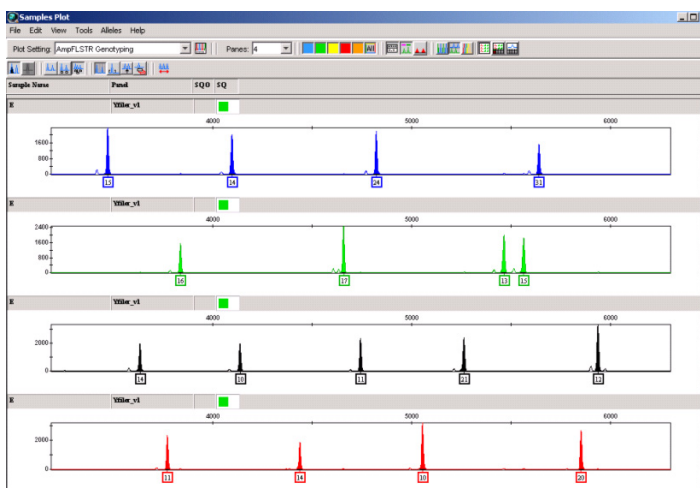


Figure 5 Align by data point

Section 3 New Features and Procedures: Yfiler Kit

This section covers:

New Features and Procedures Summary	25
Allele Calling Parameters for New Marker Repeat Types	27
Plus Stutter Filtering and –2-bp Filtering Workaround.	27
Creating HID Analysis Methods for the Yfiler Kit.	30
Creating a Table Setting and Uploading Exported Haplotype(s) . .	37

New Features and Procedures Summary

Two features have been added in GeneMapper *ID* Software v3.2 to facilitate analysis of the AmpF ℓ STR[®] Yfiler[™] PCR Amplification Kit:

- Allele calling parameters for new marker repeat types
- Plus stutter filtering

Additionally, Applied Biosystems has developed several procedures for using GeneMapper *ID* Software v3.2 with the Yfiler kit:

- Workaround for the DYS19 locus (–2-bp filtering)
- Creating HID analysis methods for the Yfiler kit
- Exporting haplotypes from GeneMapper *ID* Software v3.2

[Table 2 on page 26](#) provides a brief summary of these new features and procedures. For more detailed information, see the pages referenced in the table.

Table 2 New features and procedures summary

Feature/Procedure	Description	See Page
New feature: Allele calling parameters for new marker repeat types	In GeneMapper ID Software v3.1, allele calling parameters were only available for markers with tetranucleotide repeat motifs. In GeneMapper ID Software v3.2, however, allele calling parameters are available for four marker repeat types: tri-, tetra-, penta-, and hexanucleotide. The four allele calling parameters appear in the Allele tab of the Analysis Method Editor (as shown in Figure 6 on page 29). You may enter all related analysis values directly into the Tri, Tetra, Penta, or Hexa column fields, which allows you to see all values at once.	27
New feature: Plus stutter filtering (for the DYS392 locus)	To aid in interpreting genotype profiles, two new fields have been added to the Allele tab of the Analysis Method Editor specifically to filter out the DYS392 plus stutter: • Plus Stutter Ratio • Plus Stutter Distance	27
Procedure: Workaround for the DYS19 locus (–2 bp filtering)	Laboratories may choose to implement a workaround for the DYS19 locus by using the Minus A Ratio and Minus A Distance fields in the Allele tab of the Analysis Method Editor. These fields can be used to filter out the –2-bp stutter that is observed at the DYS19 locus.	
Procedure: Creating HID analysis methods for the Yfiler kit	Use the Analysis Method Editor in GeneMapper ID Software v3.2 to set analysis parameter values for analyzing the Yfiler kit data.	30
Procedure: Creating a table setting and uploading exported haplotype(s) for searching profiles with the Yfiler™ Haplotype Database	Using the Table Setting Editor, you can create a table setting in GeneMapper ID Software v3.2 to export haplotypes specifically for searching the Yfiler Haplotype Database for profile match estimation.	37

Allele Calling Parameters for New Marker Repeat Types

Peak Detection Analysis Parameters

The peak detection analysis parameters allow you to specify the minimum peak height to be detected for analysis. This, in turn, controls the number of peaks analyzed. Peaks falling below the parameters specified are displayed in the electropherogram, but are not labeled and will not be genotyped.

In the Allele tab of the Analysis Method Editor, a number of parameters can be set to control allele calling, including:

- Bin set
- Marker-specific stutter ratio
- Amelogenin cutoff
- Marker repeat type

New Marker Repeat Types

In GeneMapper *ID* Software v3.1, allele calling parameters were only available for markers with tetranucleotide repeat motifs. In GeneMapper *ID* Software v3.2, however, allele calling parameters are available for four marker repeat types: tri-, tetra-, penta-, and hexanucleotide.

The four allele calling parameters appear in the Allele tab of the Analysis Method Editor (as shown in [Figure 6 on page 29](#)). You may enter all related analysis values directly into the Tri, Tetra, Penta, or Hexa column fields, which allows you to see all values at once.

Plus Stutter Filtering and –2-bp Filtering Workaround

The following have been reported:

- Plus stutter at the DYS392 locus
- –2-bp stutter at the DYS19 locus

Further descriptions of these loci are provided on [page 28](#). Allele calling parameters that can be used as workarounds are provided on [page 28](#).

Plus Stutter (DYS392)

The DYS392 trinucleotide repeat locus displays the typical –3-bp stutter but also a larger +3-bp stutter. Sequencing analysis of this +3-bp stutter revealed that the product contains an additional repeat unit relative to the true allele peak.

Based on developmental validation studies conducted by Applied Biosystems, the highest percent stutter observed for any DYS392 allele was 7.9%.

–2-bp DYS19 Stutter

It has been reported that the DYS19 tetranucleotide repeat locus displays the typical –4-bp stutter but also a smaller –2-bp stutter.

Based on developmental validation studies conducted by Applied Biosystems, the highest percent stutter observed for any DYS19 locus was 10.21%.

Allele Calling Parameters

The Allele tab of the Analysis Method Editor is used to set allele calling parameters for data processing.

Select the **Use marker-specific stutter ratio if available** checkbox. When this checkbox is selected, the software uses the stutter ratios defined in the panel. These values are provided in the panels supplied by Applied Biosystems.

To filter out the reproducible DYS19 tetranucleotide stutter, the Minus A Ratio and Minus A Distance fields can be used. Additionally, two new fields specifically developed to filter out the DYS392 trinucleotide repeat plus stutter have been added to the Analysis Method Editor: Plus Stutter Ratio and Plus Stutter Distance.

[Figure 6 on page 29](#) shows allele calling parameters that can be used with GeneMapper ID Software v3.2.

Analysis Method Editor - HID

General | **Allele** | Peak Detector | Peak Quality | Quality Flags

Bin Set: AmpFLSTR_Yfiler_Binset_v1

☒ Use marker-specific stutter ratio if available

Marker Repeat Type :		Tri	Tetra	Penta	Hexa
Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.1021	0.0	0.0
MinusA Distance	From	0.0	1.5	0.0	0.0
	To	0.0	2.5	0.0	0.0
Minus Stutter Ratio		0.0	0.0	0.0	0.0
Minus Stutter Distance	From	2.25	3.25	4.25	5.25
	To	3.75	4.75	5.75	6.75
Plus Stutter Ratio		0.079	0.0	0.0	0.0
Plus Stutter Distance	From	2.25	0.0	0.0	0.0
	To	3.75	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Range Filter... Factory Defaults

OK Cancel

Figure 6 Allele calling parameters

Note: The circled values shown in [Figure 6](#) above are based on developmental validation studies conducted at Applied Biosystems and should only be used as reference values. Each laboratory should perform their own internal validation studies to determine the appropriate values to enter. The DYS19 workaround is not locus-specific. All peaks for the tetranucleotide loci falling within the range specified in the Minus A Ratio and Minus A Distance fields will be filtered.

Creating HID Analysis Methods for the Yfiler Kit

Use the Analysis Method Editor to set analysis parameter values for analyzing the Yfiler kit data.

Note: The size distribution for the Yfiler kit is ~100 to 330 bp. As a result, the 450-bp size standard peak does not need to be included in the size standard definition for analysis.

Workflow

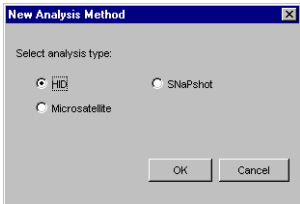
1. Import the Yfiler kit panels and bin sets.

See the *GeneMapper™ ID Software Version 3.1 Human Identification Analysis Tutorial* (PN 4335523) for procedures on importing panels and bin sets.

2. Create a new HID_Advanced analysis method for use with the AmpFISTR Yfiler kit (below).
3. Create a new HID_Classic analysis method for use with the AmpFISTR Yfiler kit ([page 36](#)).

Create the HID_Advanced Analysis Method

To create the HID_Advanced analysis method:

1.	Select Tools > GeneMapper Manager to open the GeneMapper Manager.
2.	Select the Analysis Methods tab, then click New to open the New Analysis Method dialog box. 
3.	Select HID , then click OK to open the Analysis Method Editor with the General tab selected.
4.	Select the analysis method settings shown in Table 3a on page 31 through Table 3e on page 35 . IMPORTANT! You must select your settings on all the tabs before you click OK to save the analysis method and return to GeneMapper Manager!

Analysis Method Settings

IMPORTANT! The values shown in [Table 3b on page 32](#) through [Table 3e on page 35](#) are based on developmental validation studies conducted at Applied Biosystems and should only be used as reference values. Each laboratory should perform their own internal validation studies to determine the appropriate values to enter.

Table 3a Analysis method settings: General tab

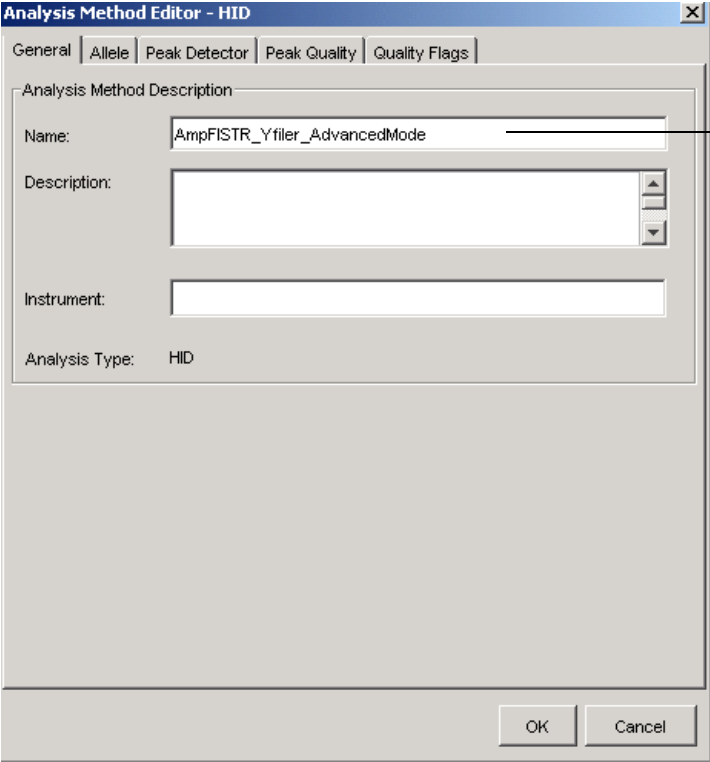
Tab	Settings
General	
Notes for the General tab: - Enter a name of your choosing. For example, enter: AmpFISTR_Yfiler_AdvancedMode, if you are creating an HID_Advanced analysis method, or AmpFISTR_Yfiler_ClassicMode, if you are creating an HID_Classic analysis method - Select your instrument from the drop-down list.	

Table 3b Analysis method settings: Allele tab

Tab	Settings																																																							
Allele	Analysis Method Editor - HID General Allele Peak Detector Peak Quality Quality Flags Bin Set: AmpFLSTR_Yfiler_Binset_v1 ☒ Use marker-specific stutter ratio if available <table><thead><tr><th>Marker Repeat Type :</th><th>Tri</th><th>Tetra</th><th>Penta</th><th>Hexa</th></tr></thead><tbody><tr><td>Cut-off Value</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr><tr><td>MinusA Ratio</td><td>0.0</td><td>0.1021</td><td>0.0</td><td>0.0</td></tr><tr><td>MinusA Distance</td><td>From 0.0</td><td>1.5</td><td>0.0</td><td>0.0</td></tr><tr><td></td><td>To 0.0</td><td>2.5</td><td>0.0</td><td>0.0</td></tr><tr><td>Minus Stutter Ratio</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr><tr><td>Minus Stutter Distance</td><td>From 2.25</td><td>3.25</td><td>4.25</td><td>5.25</td></tr><tr><td></td><td>To 3.75</td><td>4.75</td><td>5.75</td><td>6.75</td></tr><tr><td>Plus Stutter Ratio</td><td>0.079</td><td>0.0</td><td>0.0</td><td>0.0</td></tr><tr><td>Plus Stutter Distance</td><td>From 2.25</td><td>0.0</td><td>0.0</td><td>0.0</td></tr><tr><td></td><td>To 3.75</td><td>0.0</td><td>0.0</td><td>0.0</td></tr></tbody></table> Amelogenin Cutoff 0.0 Range Filter... Factory Defaults OK Cancel	Marker Repeat Type :	Tri	Tetra	Penta	Hexa	Cut-off Value	0.0	0.0	0.0	0.0	MinusA Ratio	0.0	0.1021	0.0	0.0	MinusA Distance	From 0.0	1.5	0.0	0.0		To 0.0	2.5	0.0	0.0	Minus Stutter Ratio	0.0	0.0	0.0	0.0	Minus Stutter Distance	From 2.25	3.25	4.25	5.25		To 3.75	4.75	5.75	6.75	Plus Stutter Ratio	0.079	0.0	0.0	0.0	Plus Stutter Distance	From 2.25	0.0	0.0	0.0		To 3.75	0.0	0.0	0.0
Marker Repeat Type :	Tri	Tetra	Penta	Hexa																																																				
Cut-off Value	0.0	0.0	0.0	0.0																																																				
MinusA Ratio	0.0	0.1021	0.0	0.0																																																				
MinusA Distance	From 0.0	1.5	0.0	0.0																																																				
	To 0.0	2.5	0.0	0.0																																																				
Minus Stutter Ratio	0.0	0.0	0.0	0.0																																																				
Minus Stutter Distance	From 2.25	3.25	4.25	5.25																																																				
	To 3.75	4.75	5.75	6.75																																																				
Plus Stutter Ratio	0.079	0.0	0.0	0.0																																																				
Plus Stutter Distance	From 2.25	0.0	0.0	0.0																																																				
	To 3.75	0.0	0.0	0.0																																																				
Notes for the Allele tab: - Be sure to select the Use marker-specific stutter ratio if available checkbox. When this checkbox is selected: - The software uses the stutter ratios defined in the panel. - The stutter ratios include all loci except for the DYS392 +3 stutter and the DYS19 –2 stutter. The stutter ratios are defined in the Analysis Method Editor under the Allele tab, if applicable. - See “Plus Stutter Filtering and –2-bp Filtering Workaround” on page 27 for a discussion of the Tetra column values in the Minus A Ratio/Distance fields and Tri column values in the Plus Stutter Ratio/Distance fields.																																																								

Table 3c Analysis method settings: Peak Detector tab

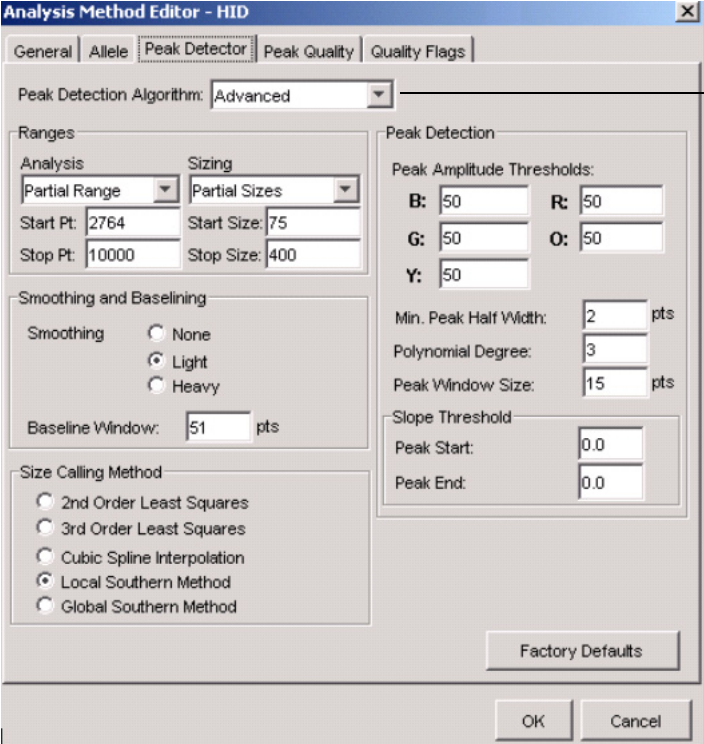
Tab	Settings
Peak Detector	 Select Advanced or Classic here, depending on the analysis method you are creating. See the <i>Notes</i> below.
Notes for the Peak Detector tab: From the Peak Detection Algorithm drop-down list, select: • Advanced, if you are creating an HID_Advanced analysis method, or • Classic, if you are creating an HID_Classic analysis method	

Table 3d Analysis method settings: Peak Quality tab

Tab	Settings
Peak Quality	Analysis Method Editor - HID General Allele Peak Detector Peak Quality Quality Flags Signal level Homozygous min peak height 200.0 Heterozygous min peak height 100.0 Heterozygote balance Min peak height ratio 0.7 Peak morphology Max peak width (basepairs) 1.5 Pull-up peak Pull-up ratio 0.05 Allele number Max expected alleles 2 Factory Defaults OK Cancel

Notes for the Peak Quality tab:

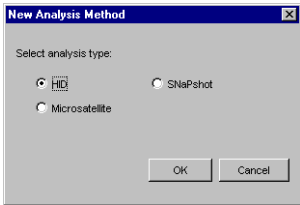
The values shown in the Peak Quality tab have been chosen based on empirical data generated at Applied Biosystems and should only be used as reference values. Each laboratory should perform their own internal validation studies to determine the appropriate values to enter.

Table 3e Analysis method settings: Quality Flags tab

Tab	Settings
Quality Flags	Analysis Method Editor - HID General Allele Peak Detector Peak Quality Quality Flags Quality weights are between 0 and 1. Quality Flag Settings Spectral Pull-up 0.8 Broad Peak 0.8 Out of Bin Allele 0.8 Overlap 0.8 Control Concordance 1.0 Low Peak Height 0.3 Off-scale 0.8 Peak Height Ratio 0.3 PGV Thresholds Pass Range: Low Quality Range: Sizing Quality: From 0.75 to 1.0 From 0.0 to 0.25 Genotype Quality: From 0.75 to 1.0 From 0.0 to 0.25 Factory Defaults OK Cancel
Notes for the Quality Flags tab: The weighted values in the Quality Flag Settings section were ranked on a scale from most important to least important. This ranking was performed by the Applied Biosystems HID Group.	

Create the HID_Classic Analysis Method

To create the HID_Classic analysis method:

1.	Select Tools > GeneMapper Manager to open the GeneMapper Manager.
2.	Select the Analysis Methods tab, then click New to open the New Analysis Method dialog box. 
3.	Select HID , then click OK to open the Analysis Method Editor.
4.	Select the analysis method settings shown in Table 3a on page 31 through Table 3e on page 35 . IMPORTANT! You must select your settings on all the tabs before you click OK to save the analysis method and return to GeneMapper Manager!

What Next?

After creating analysis methods for the Yfiler kit, continue with data analysis as follows:

1. Import sample files
2. Select analysis parameters
3. Analyze the project

For More Information

For more information, refer to the following documents:

- *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775)
- *GeneMapper™ ID Software Version 3.1 Human Identification Analysis Tutorial* (PN 4335523)
- *AmpFISTR® Yfiler™ PCR Amplification Kit User's Manual* (PN 4358101)

Creating a Table Setting and Uploading Exported Haplotype(s)

Using the Table Setting Editor, you can create a table setting in GeneMapper *ID* Software v3.2 to export haplotypes specifically for searching the Yfiler™ Haplotype Database for profile match estimation.

Database Online Search Tool

The Yfiler Haplotype Database online search tool searches for haplotypes generated with the Yfiler kit. The online search tool allows you to:

- Estimate the frequency of a given Y-chromosome haplotype in specified populations. (Frequency calculations are based on the haplotype data generated from the 17 loci included in the Yfiler kit. This data was compiled from more than 3000 samples from a range of populations.)
- Estimate the frequency of haplotypes in a number of reference populations.
- Search complete or partial profiles generated with the Yfiler kit.
- Compare the discrimination capacity of the Yfiler kit relative to the recombination of Ystr loci.

Workflow

1. Use the Table Setting Editor to create a table setting in GeneMapper *ID* Software v3.2 (below).
2. Upload the exported haplotype(s) into the Yfiler Haplotype Database for profile match estimation ([page 40](#)).

Create a Table Setting

Define custom Samples and Genotypes views for displaying the Yfiler kit data.

To create a table setting:

1.	From the GeneMapper Manager , select the Table Settings tab.
2.	Click New to open the Table Setting Editor.

To create a table setting: *(continued)*

3. Select the **General** tab and complete it as follows:
 - a. Enter a **Name** of your choosing.
 - b. If desired, enter a **Description**.

Table Setting Editor

General | Samples | Genotypes

Table Settings Description:

Name: AmpFSTR_Yfiler_Upload_Table

Description:

OK Cancel

4. Select the **Samples** tab and complete it as follows:
 - a. In the Column Settings section, deselect all options.
 - b. In the Font Settings section, leave the default values.

Table Setting Editor

General | Samples | Genotypes

Samples Table Settings:

Column Settings:

	Show	Column	Filtering	Content
1	☐	Status	Show All Records	N/A
2	☐	Sample File	Show All Records	
3	☐	Sample Name	Show All Records	
4	☐	Sample ID	Show All Records	
5	☐	Comments	Show All Records	
6	☐	Sample Type	Show All Records	N/A
7	☐	Specimen Category	Show All Records	N/A
8	☐	Analysis Method	Show All Records	

Font Settings:

Font: Arial

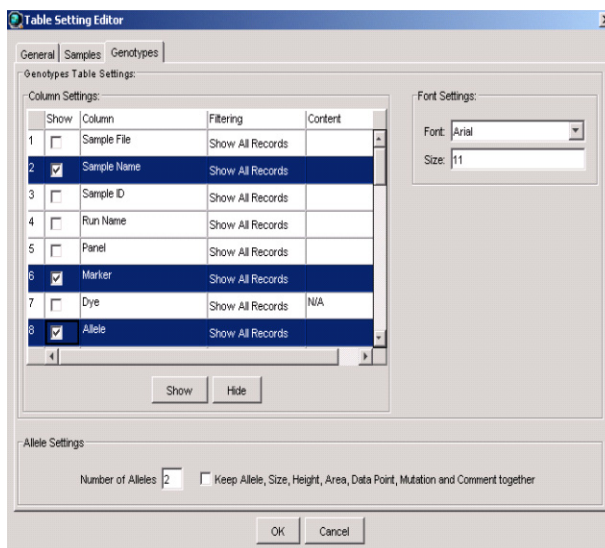
Size: 11

Show Hide

OK Cancel

To create a table setting: *(continued)*

5. Select the **Genotypes** tab and complete it as follows:
 - a. In the Column Settings section, select:
 - **Sample Name**
 - **Marker**
 - **Allele**
 Deselect all of the other options.
 - b. In the Font Settings section, leave the default values.
 - c. In the Allele Settings section:
 - Enter **2** in the Number of Alleles field.
 - Deselect the **Keep Allele, Size...** check box.



6. Click **OK** to save the table settings, close the Table Setting Editor, and return to the GeneMapper Manager.

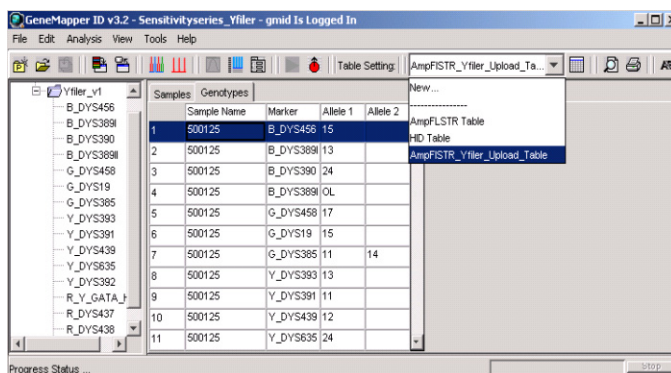
Upload the Exported Haplotype(s)

Upload the exported haplotype(s) into the Yfiler Haplotype Database.

To upload the exported haplotype(s):

1. If you are not already there, open the project in GeneMapper ID Software v3.2.

2. From the **Table Settings** drop-down list, select the table setting you created specifically to export haplotype(s) for searching the Yfiler Haplotype Database.



3. For the remaining upload procedures, see:
 - The Yfiler Haplotype Database:
<http://www.appliedbiosystems.com/yfilerdatabase/>
or
 - The *AmpFISTR® Yfiler™ PCR Amplification Kit User's Manual* (PN 4358101)

Appendix A Verification Testing

This appendix covers:

Introduction	42
Materials and Methods	43
Category 1: Peak Detection and Genotyping (Reproducibility) . . .	45
Category 2: Algorithm Testing	46
Category 3: Data Handling	48
Category 4: Workflow	48
Results for Category 1: Peak Detection and Genotyping (Reproducibility)	49
Results for Category 2: Algorithm Testing	53
Results for Category 3: Data Handling	54
Results for Category 4: Workflow	55
Results Summary	55

Introduction

Test Plan The test plan, which Applied Biosystems has defined as verification of the software, was designed to evaluate the performance of GeneMapper *ID* Software v3.2 for the human identification communities.

Test Categories Applied Biosystems performed verification of GeneMapper *ID* Software v3.2 to assess the performance, robustness, and feature design in four specific categories:

- Peak Detection and Genotyping (Reproducibility) ([page 45](#))
- Algorithm Testing ([page 46](#))
- Data Handling ([page 48](#))
- Workflow ([page 48](#))

Evaluation GeneMapper *ID* Software v3.2 verification was performed to:

- Confirm and document the functionality of the new software features
- Assess the functionality of the minor modifications made to the analysis method

Our findings demonstrate that GeneMapper *ID* Software v3.2 (with its default settings and panels and bin sets) is valid for forensic, paternity, and databasing analyses. GeneMapper *ID* Software v3.2 accurately detects, genotypes, and performs quality checks when performing STR analysis.

Materials and Methods

This section describes the AmpF ℓ STR[®] kit, sample types, instruments, and software used for the GeneMapper[®] ID Software v3.2 verification testing.

Kit The AmpF ℓ STR[®] Yfiler[™] PCR Amplification Kit was used to amplify each sample type for the verification testing.

- Sample Types**
- Single-source DNA Samples – Eighty (80) DNA samples from four (4) population groups
 - Mixture Samples – Mixtures of male and male DNA were amplified. These samples were mixed in defined ratios of 1:1, 3:1, 10:1, and 15:1, respectively. Additionally, mixtures of male and female DNA were amplified. These samples were mixed in defined ratios of 1:1000, 1:2000, 1:4000, and 1:8000, respectively.

Note: The human genomic DNA samples were quantified using Quantifiler[™] Human DNA Quantification Kit and Quantifiler[™] Y Human Male DNA Quantification Kit. The samples were amplified using a GeneAmp[®] PCR System 9700 with a silver 96-well block. The recommended cycling conditions were used, as outlined in the *AmpF ℓ STR[®] Yfiler[™] PCR Amplification Kit User's Manual* (PN 4358101). The amplified samples were injected on three or more ABI PRISM[®] instrument platforms.

Instruments and Software

[Table 4](#) below lists the instruments, Data Collection software versions, computer operating systems, and run modules that were used to process the samples for verification testing.

Table 4 Forensic validated instrument platforms

Instrument	Data Collection Software	Computer Operating System	Run Module
ABI PRISM® 310 Genetic Analyzer	v3.0	Microsoft® Windows NT®	• GS STR Pop4 (1 ml) F • GS STR Pop4 (1 ml) G5v2
ABI PRISM® 3100 Genetic Analyzer	v1.1	Microsoft® Windows NT®	• GS 36_Pop4_F • GS 36vb_Pop4_G5
	v2.0	Microsoft® Windows 2000	

Category 1: Peak Detection and Genotyping (Reproducibility)

The first test category evaluated genotype concordance using single-source population DNA samples, male:male mixture samples, and male:female mixture samples. All samples were amplified with the Yfiler kit.

Two comparison studies were conducted to verify genotype concordance using DNA samples previously amplified with the Yfiler kit. These data were used to compare the genotype results from data analysis with GeneScan® Software v3.7.1 and Genotyper® Software v3.7 (both running on the Windows NT operating system) to GeneMapper *ID* Software v3.2 (running on the Windows 2000 operating system) using the Advanced mode.

Single-Source Population DNA Samples

The first comparison study consisted of:

- Eighty (80) samples from the Yfiler kit population study
- Four (4) positive controls
- One (1) negative control
- Six (6) ladders

These samples were electrophoresed on the ABI PRISM® 3100 Genetic Analyzer and consisted of 1222 alleles from the samples, 60 alleles from the controls, and 137 alleles from the ladders. The samples were examined for reproducibility.

Mixture Samples

The second comparison study consisted of mixture data produced during the Yfiler kit developmental validation. This study was conducted to verify that the same mixture samples (that is, PCR product) gave concordant genotype calls between the GeneScan/Genotyper software and the GeneMapper *ID* software applications.

The mixture samples were electrophoresed on three different instruments and consisted of five (5) male:male and five (5) male:female mixture samples. [Table 5](#) below lists the mixture ratios that were used in the mixture experiment.

Table 5 Mixture ratios

Male:Male	Male:Female
1:1	1:1000
3:1	1:2000
10:1	1:4000
15:1	1:8000

Category 2: Algorithm Testing

The second test category evaluated the functionality of GeneMapper *ID* Software v3.2 to accurately filter out stutter based on the Marker Specific Stutter ratio specified in the panel and in the Allele tab of the Analysis Method Editor.

Stutter Evaluation

In the Allele tab of the Analysis Method Editor, one of the parameters that can be set to filter out stutter is the **Use marker-specific stutter ratio if available** check box.

Each allele within a locus displays a percent stutter that is reproducible. The **Use marker-specific stutter ratio if available** check box was selected in order to use the stutter ratio information from the panel. Using GeneMapper *ID* Software v3.2, 27 population samples were examined and the percent stutter recorded. The 27 population samples consisted of:

- 432 (n-4), (n-5), and (n-6) stutter peaks
- 27 DYS19 (n-2) stutter peaks
- 27 DYS392 (n+3) stutter peaks

Spectral Pull-Up PQV

Laboratories that have implemented the ABI PRISM 3100 Genetic Analyzers into their workflow to electrophorese DNA samples may observe pull-up peaks within ± 2 data points (in a different color) from the main allele peak, which is causing the pull-up peak. To this end, Applied Biosystems has made modifications to the analysis method to flag spectral pull-up peaks in the range of ± 2 data points.

The spectral pull-up PQV flag was evaluated to ensure that a peak meeting specified criteria for spectral pull-up is appropriately activated. The two criteria required to flag a peak as spectral pull-up are as follow:

- The peak is $\leq 5\%$ (or as specified by the value in the **Pull-up ratio** field in the Peak Quality tab of the Analysis Method Editor) of the main allele peak causing the pull-up
- The peak is within the ± 2 data points range from the main allele peak in a different color

Note: After installing GeneMapper *ID* Software v3.2, laboratories should create new analysis methods specifically for use with GeneMapper *ID* Software v3.2. Creating new analysis methods will ensure that spectral pull-up peaks within ± 2 data points from the main allele peak are flagged appropriately.

Spectral Pull-UP PQV Study

To test the functionality of the spectral pull-up PQV, twenty-eight (28) samples from the single-source population study were electrophoresed on both the ABI PRISM[®] 310 and 3100 Genetic Analyzers (that is, the same PCR product was electrophoresed on two different instruments) and were evaluated using the following criteria:

- Each analyzed sample was evaluated for the presence of pull-up peaks by noting whether or not the spectral pull-up PQV was flagged.
- The data point (that is, ± 0 data point, ± 1 data point, or ± 2 data points) of the pull-up peak relative to the main allele peak was recorded.
- The percent height of the pull-up peak relative to the main allele peak was recorded (that is $\leq 5\%$ of the main allele peak causing the pull-up)

Category 3: Data Handling

The third test category evaluated the Export Combined Table format and the size standard printing functionality.

Export Combined Table Format

The purpose for evaluating the Export Combined Table format was to verify that samples that do not pass sizing (that is, low-quality samples) are exported along with samples that pass sizing.

Printing Labeled Size Standard Peaks

In GeneMapper *ID* Software v3.2, the ability to display and print labels on the size standards in different plot configurations was tested.

Category 4: Workflow

The fourth test category evaluated the ability of GeneMapper *ID* Software v3.2 to:

- Display labeled peak assignments for all size standards
- Retain labels in both the “align by base pair” and “align by data point” views for the X-axis

Displaying Labeled Peak Assignments for Size Standards

All samples within the Samples view were highlighted and only the orange dye color was selected, as shown below.



The size standards were checked to ensure that all defined size standard peaks were labeled appropriately.

Retaining Labels in Base Pair and Data Point Views

The electropherogram plots were displayed to verify that labels associated with the peaks were retained when switching back and forth between the “align by base pair” and “align by data point” views for the X-axis.

Results for Category 1: Peak Detection and Genotyping (Reproducibility)

Single-Source Population DNA Samples

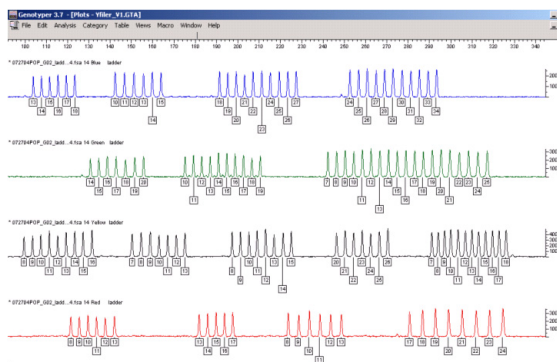
The first parameter evaluated the Peak Detection and Genotyping category to assess genotyping concordance. Applied Biosystems compared the genotype results from data analysis with GeneScan Software v3.7.1 and Genotyper Software v3.7 (both running on the Windows NT operating system) to GeneMapper *ID* Software v3.2 using the Advanced algorithm.

The analysis parameter settings for the software packages were defined according to Applied Biosystems default analysis parameters with a defined peak amplitude threshold (PAT) of 50 RFUs.

As shown in [Figure 1](#) below, a side-by-side comparison between Genotyper Software v3.7 and GeneMapper *ID* Software v3.2 was evaluated to ensure the consistency of genotype results, where the evaluation criteria was that all samples give concordant genotypes.

After the trained scientist completed data analysis, each population sample tested gave 100% concordant genotype calls between the two software programs (see [Table 6 on page 50](#)).

Genotyper Software v3.7



GeneMapper *ID* Software v3.2

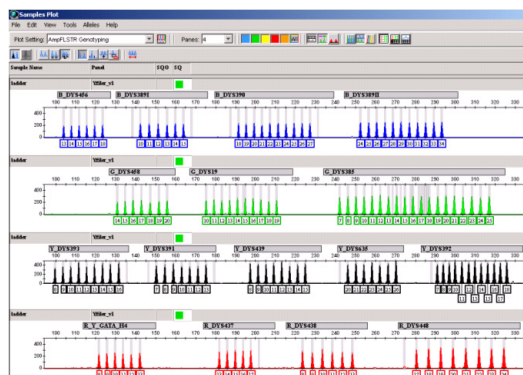


Figure 1 Example of the Yfiler kit allelic ladder analyzed with Genotyper Software v3.7 (left) and GeneMapper *ID* Software v3.2 displaying concordant results (right)

As shown in [Table 6](#) below, each sample type tested gave concordant genotype calls between the two software programs.

Table 6 Genotype concordance between Genotyper Software v3.7 and GeneMapper ID Software v3.2

Allele Source	Number of Samples	Number of Alleles	Concordant Alleles
Population samples	80	1222	100%
Positive and negative controls	5	60	100%
Allelic ladders	6	137	100%

Mixture Samples

After the trained scientist completed data analysis, Applied Biosystems documented three observations when comparing the results produced from GeneScan Software v3.7.1 and Genotyper Software v3.7 (both running on the Windows NT operating system) to the results produced from GeneMapper ID Software v3.2. Of the 272 loci examined, 267 loci produced concordant results between the software packages and 5 loci/alleles produced non-concordant results. These observations are further described on [page 51](#).

[Figure 2](#) below shows the genotyping concordance results when comparing the analyzed data from GeneScan Software v3.7.1 and Genotyper Software v3.7 (both running on the Windows NT operating system) to the results from GeneMapper ID Software v3.2

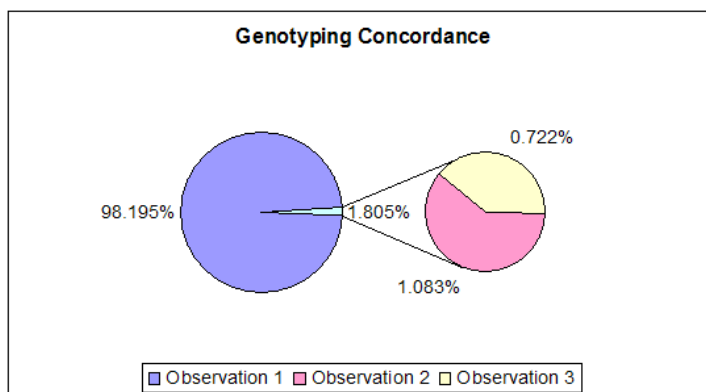


Figure 2 Genotyping concordance results

- **Observation 1:** 267 (98.20%) of the loci/alleles compared produced concordant genotype calls.
- **Observation 2:** Three (1.08%) of the loci/alleles were non-concordant because these loci/alleles fell just below the 50-RFU cutoff threshold value in GeneMapper *ID* Software v3.2 and were not genotyped.
- **Observation 3:** Two (0.72%) of the loci/alleles were non-concordant because the alleles analyzed with Genotyper Software v3.7 did not filter the stutter or the Minus A. The label is removed from Peak A (the stutter peak) if Peak B (the true allele) meets two criteria:
 - Peak B is higher than Peak A by the specified percentage, *and*
 - Peak B is within the specified proximity size (in base pairs) range relative to Peak A

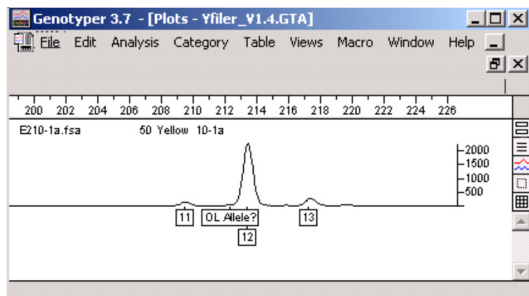
[Table 7](#) below shows the results for observation 3.

Table 7 Observation 3: non-concordant loci

Marker/Locus	Genotyper Software v3.7				GeneMapper <i>ID</i> Software v3.2	
	Allele 1	Allele 2	Allele 3	Allele 4	Allele 1	Allele 2
DYS439	11	OL	12	13	12	13
DYS456	15	16	OL	17	15	17

As shown in [Figure 3](#) and [Figure 4 on page 52](#), these two loci/alleles did not meet these criteria due to –A product, resulting in the detection of a third peak (Peak C). The filtering in Genotyper Software v3.7 compared Peak A to Peak C. GeneMapper *ID* Software v3.2 appropriately filtered the stutter and the shoulder peak using defined bin sets

Genotyper Software v3.7



GeneMapper ID Software v3.2

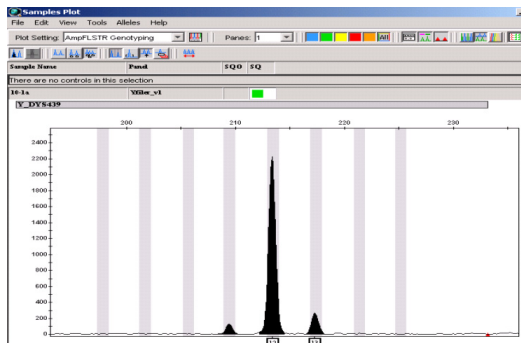
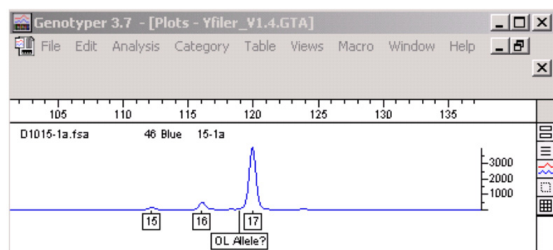


Figure 3 DYS439 Marker

Genotyper Software v3.7



GeneMapper ID Software v3.2

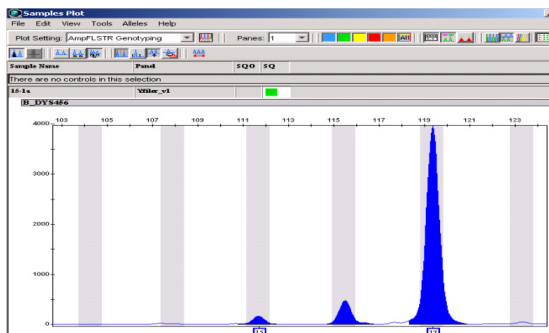


Figure 4 DYS456 Marker

Results for Category 2: Algorithm Testing

Stutter Evaluation Twenty-seven (27) population DNA samples were examined, consisting of:

- 432 (n-4), (n-5), and (n-6) stutter peaks
- 27 DYS19 (n-2) stutter peaks
- 27 DYS392 (n+3) stutter peaks

The results were as follows:

- All (n-4, n-5, and n-6) stutter peaks were below the stutter percent as defined in the panel and were properly filtered.
- The DYS19 locus (n-2) stutter peak was below the stutter percent as defined in the Allele tab of the Analysis Method Editor and were properly filtered.
- The DYS392 locus (n+3) stutter peak was below the stutter percent as defined in the Allele tab of the Analysis Method Editor and were properly filtered.

Spectral Pull-Up PQV Twenty-eight (28) single-source population samples were evaluated for spectral pull-up. For each pull-up peak, the following was recorded:

- Size
- Peak height
- Data point
- Spectral pull-up PQV activated

Seventy-eight (78) spectral pull-up peaks were identified. As shown in [Figure 5 on page 54](#), 97.44% (76) of these pull-up peaks were correctly flagged by GeneMapper *ID* Software v3.2. The remaining 2.56% (2) of pull-up peaks that were not flagged were produced by off-scale data. If a spectral pull-up peak is caused by an off-scale allele peak, the spectral pull-up flag is not triggered for that peak. This is because the ratio of pull-up to off-scale peaks cannot be calculated (since the peak height for off-scale data cannot be determined).

Applied Biosystems recommends that you carefully review all off-scale yellow PQV flags. Applied Biosystems recommends that you dilute or re-run samples to obtain on-scale data to ensure the PQVs reported by data analysis are accurate and to reduce spectral separation (that is, pull-up).

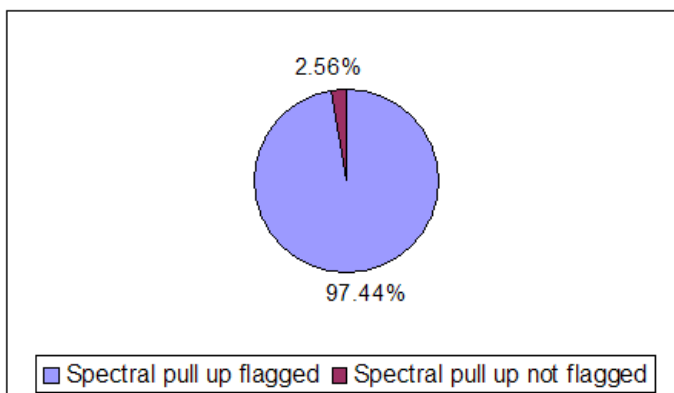


Figure 5 Results for the spectral pull-up PQVs flagged

Results for Category 3: Data Handling

Export Combined Table Format

Projects with their associated settings (that is, analysis methods, table settings, plot settings, matrices, if applicable, and size standards) consisting of samples that passed sizing as well as samples that did not pass sizing were exported. The tables were exported from the Samples view using the Export Combined Table format. Eighty (80) samples from the Yfiler kit population study were used.

The results obtained verified that GeneMapper ID Software v3.2 is 100% functional in its ability to:

- Export projects using the Export Combined Table format
- Track all samples in an electrophoresis run (samples that do not pass sizing as well as samples that pass sizing)

Printing Labeled Size Standard Peaks	The purpose of this test was to verify that the size standard plots could be printed in different display configurations. The results obtained verified that GeneMapper <i>ID</i> Software v3.2 is 100% functional in its ability to display and print labels on size standards in different plot configurations. For example, displaying five labeled size standards separated into five panes, using three levels of magnification.
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Results for Category 4: Workflow

Displaying Labeled Peak Assignments	All size standards from eighty (80) DNA samples from the Yfiler kit single-source population study were examined. All peaks were correctly labeled.
Retaining Labels	The results obtained verified that GeneMapper <i>ID</i> Software v3.2 is 100% functional in its ability to switch back and forth between the “align by base pair” and “align by data point” views for the X-axis.

Results Summary

Applied Biosystems verification testing demonstrates that human identification laboratories can successfully adopt GeneMapper *ID* Software v3.2.

Appendix B Troubleshooting the Installation

This appendix covers:

Troubleshoot the Installation.	58
Uninstall the Software.	59
Troubleshooting Checklist.	61
How to Obtain Support	63

Troubleshoot the Installation

Workflow If the installation appears to be successful, but the GeneMapper *ID* software fails to start and reports the error “Failed to connect to database,” perform the troubleshooting procedures in this appendix in the order shown below.

1. Check the Oracle® database installation ([page 58](#)).
2. Configure the Oracle database manually ([page 58](#)).

Check the Oracle Database Installation

Check to see if the Oracle database instance was installed correctly.

To check the Oracle database installation:

1. Select **Start > Programs > Accessories > Command Prompt**.
2. Type **sqlplus**, then press **Enter**. You should see:
SQL*Plus: Release 8.1.7.0.0...
3. Type the user name **system**, then press **Enter**.
4. Type the password **manager**, then press **Enter**. If:
 - A “Connected to:” message is displayed, the Oracle database was installed correctly. Proceed with “[Configure the Database Manually](#)” on [page 58](#).
 - If the Oracle database was not installed correctly, an error message appears. Complete [Table 8, “Troubleshooting Checklist,”](#) on [page 61](#), then contact Technical Support.

Configure the Database Manually

If the Oracle database was installed correctly, perform the following steps in the Command Prompt window to configure the database manually.

To configure the database:

1. Return to the open Command Prompt window (it should still be open from [step 4](#) above).
2. At the SQL prompt, type **exit** to exit the SQL Plus application.
3. At the drive prompt, type **cd /d x:\GeneMapper\database**, where *x* is the drive where the GeneMapper *ID* software is installed, then press **Enter**.

4. At the drive prompt, type **ifadb x:\oraclegm\oradata\ifa**, where *x* is the drive where the GeneMapper *ID* software is installed, then press **Enter**.
 - If “Succeeded” messages are displayed, the database is configured correctly. Proceed to [“Start the Software” on page 14](#).
 - If “Error” messages are displayed, the database is not configured correctly. Proceed to [“Uninstall the Software” on page 59](#).

Uninstall the Software

If the software installation is not successful and the [“Troubleshoot the Installation”](#) procedures on [page 58](#) have not resolved the problem, you may need to uninstall the GeneMapper *ID* software, then contact Technical Support.

Workflow

1. Log onto the local domain of your computer as a user with Administrator privileges ([page 59](#)).
2. Uninstall the software ([page 59](#)).
3. Complete the Troubleshooting Checklist, then contact Technical Support ([page 61](#)).

Log Onto the Computer

To uninstall the GeneMapper *ID* software, you must:

- Log onto the local computer (not a network domain)
- Have Administrator privileges on the local computer (that is, have complete and unrestricted access to the local computer)

Uninstall the Software

This procedure is for a standalone configuration only. This procedure removes the GeneMapper *ID* Software v3.2 and the Oracle Database Standard Edition v8.1.7.

To uninstall the software:

1. Save and close all applications (including the GeneMapper *ID* software) and windows before proceeding with the uninstall.
2. Select **Start > Settings > Control Panel**.
3. Double-click **Add or Remove Programs**.

4. Select **GeneMapper ID v3.2**, then click **Change/Remove**.
5. In the Welcome window, select **Remove**, then click **Next**.
6. At the prompt, click **OK** to confirm the uninstall. The uninstaller removes all GeneMapper *ID* Software v3.2 and Oracle Database Standard Edition v8.1.7 files and settings from the computer.
7. When the uninstall is complete, the Maintenance Complete page opens. Select **Yes, I want to restart my computer now**, then click **Finish**.
8. Look for the **x:\GeneMapper** folder and delete it if it still exists (sometimes the folder get left behind after an uninstall).
9. Complete the [“Troubleshooting Checklist” on page 61](#).

Troubleshooting Checklist

Before you contact Applied Biosystems Technical Support for troubleshooting assistance, complete the Troubleshooting Checklist (pages 61 to 62).

Checklist **Table 8** **Troubleshooting Checklist**

Check	Information for Technical Support
	Summarize the problem:
	Have you been able to repeat the problem? If yes, list the steps that you perform: 1. 2. 3. 4. 5. 6. 7.
	Applied Biosystems personnel that you have contacted: ☐ Field Applications Specialist ☐ Field Service Engineer ☐ Technical Support ☐ Sales Representative ☐ Order Administration ☐ Other
	Computer specifications • Operating system: • Version: • Processor: • Memory: • Hard disk space: • Hard disk configuration:

Table 8 Troubleshooting Checklist (*continued*)

Check	Information for Technical Support
	Software installed ☐ Data Collection software version: ☐ Status of Data Collection Services: ☐ GeneMapper ID software version: ☐ Other Applied Biosystems software:
	Computer login information • User privileges: • Local or networked domain:
	Software configuration installed ☐ Instrument ☐ Standalone
	Instrument and instrument computer information • Model: • Data Collection software version: • Status of Data Collection Services: • Other Applied Biosystems software: • Capillary length: • Capillary lot number: • Run module: • Dye set:
	Chemistry kit or reagent, with version number:
	Be prepared to send to Technical Support: • Exported panels • Exported bins • Exported size standard definition • Exported analysis method • Sample (.fsa) files • GeneMapper_log.txt • PanelImportLog.txt • Printed results

How to Obtain Support

Be sure to complete the Troubleshooting Checklist (page 61) before contacting Technical Support.

For the latest services and support information for all locations, go to **<http://www.appliedbiosystems.com>**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

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